Amendments to the Drawings:

The attached sheets of drawings includes the addition of Figures 19-24.

REMARKS

As will be discussed in further detail below, the specification has been amended to correct the priority claim, to insert SEQ ID NOs, to correct editorial errors that have heretofore gone unnoticed and to remove drawings from the text of the specification. These drawings are now separate figures. The abstract has been amended as well.

Claims 91-142 are pending in the above-referenced application. Claims 91-111 have been canceled without prejudice. However, Applicants reserve the option to pursue the subject matter recited in claims 91-111 in subsequent continuation and/or divisional applications. Claims 112, 123 and 133 have been amended to more distinctly claim that which Applicants regard as their invention. The amendments to these claims are supported by the specification. No new matter has been added.

Furthermore, new claims 143-148 have been added to recite specific embodiments. These new claims are supported by the specification on pages 7-8, 16-18, 22 and 24.

1. Priority

It is asserted that a proper priority claim was not made in the instant application. In response, Applicants assert that a proper priority claim was indeed made. A copy of Applicants transmittal letter is attached hereto for Examiner's reference. However, there was an improper designation of the filing date and serial number of the parent application of application serial number 10/260,031. The specification has been amended accordingly. Furthermore, Applicants herewith submit a petition to claim benefit under 35 USC 120, 121, or 365(c) of a prior copending nonprovisional application or international application designating the united states of america (37 C.F.R. 1.78(a)).

2. Abstract

The abstract of the disclosure is objected to because it exceed 150 words and uses the phrase "disclose in this invention". In response, Applicants herewith submit a revised abstract. Therefore, the objection has been overcome.

3. Specification

It is asserted that on pages 25, 30-32 and 33, the specification contains a drawing. Applicants note that there are no drawings on these recited pages. The drawing on page 25 is now FIG. 19, the drawing on page 30 is now FIG. 20, the drawings on page 31are now FIGS. 21 and 22, the drawing on page 32 is now FIG. 23 and the drawing on page 43 is now FIG. 24.

In view of the amendment of the specification, Applicants assert that the objection has been overcome and should be withdrawn.

4. Claim Rejections-35 USC 102

The claims have been rejected over Schuster et al., Kacian et al. and Cleuziat et al. These are described in detail below.

5.1 Schuster et al.

Claims 91-100, 112-121 and 133-142 are rejected under 35 U.S.C. 102(b) as being anticipated by Schuster et al. (US Patent 5,169,766 December 8, 1992). Applicants traverse the rejection of all of the claims.

First, in response, claims 91-100 have been canceled in order to advance prosecution. However, Applicants do reserve the right to pursue prosecution of the subject matter recited in the canceled claims in subsequent continuation and/or divisional applications.

5.1.1 Claims 112-121

The Office Action specifically states with respect to claim 112:

> With regard to Claim 112, the claim encompass all the limitations of Claim 91 and the steps of producing a RNA/DNA hybrid as a substrate for RNase H digestion which allows for further primer binding events to occur. Schuster et al. teaches making RNA: DNA hybrid (Column 9, lines 32-33). Schuster et al. teaches the ssRNA (which is the extended promoter) is destroyed by RNase H. Further, any primers which are in the solution but did not primer to the original ssDNA would be destroyed by RNase H,

> therefore allowing for a reaction solution with only the cDNA that allows the completion.

Applicants respectfully traverse the rejection. Schuster does not contain each and every element of the claimed invention, the subject matter recited in claim 112.

Applicants note that claims 112 has been amended to recite in step (d):

> (d) digesting said substrate with RNase H to remove said ribonucleic acid segment of said primer and allow another primer binding event to occur **with said nucleic acid of interest**, thereby producing multiple copies of said nucleic acid of interest

It is Applicants view that Schuster does not teach step (d) in claim 112 as amended. Specifically, in Applicants method, the substrate is actually an extended primer bound to the nucleic acid of interest. Thus, when the ribonucleic acid segment of said primer is actually removed from the nucleic acid of interest, a binding site is actually regenerated on said nucleic acid of interest. As a result, more primer binding and extension events occur on the nucleic acid of interest. In contrast, in Schuster, RNA copies are made off of the original template and these copies do not comprise the primers themselves. As a result, Schuster et al. may show removal of primer-derived copies, but there is no removal of RNA segments from primers as required in step (d). As such in Schuster, primer binding events only take place on resultant copies and the primer is not removed from the original template. Therefore, it would not be possible to regenerate a primer binding site on the nucleic acid of interest using the method of Schuster.

Further, Applicants take issue with the assertion made in the Office Action that primers that are in solution but did not act as primers to the original ss DNA would be destroyed by RNase H. RNaseH does not use single-stranded primers as substrates. There has to be complementary binding between an RNA segment and DNA segment for recognition as a substrate and subsequent digestion o f the RNA segment. Therefore, primers containing RNA sequences will remain intact until they bind to a target molecule, the nucleic acid of interest. In the method of the present invention, the continued presence of this pool of unbound primers allows a cycling of primer and extension events with a single template molecule.

In summary, in Schuster, primer binding events only take place on resultant copies of the nucleic acid sequence of interest, not as in the method of the present

invention the nucleic acid sequence of interest. This is because RNaseH requires the presence of an RNA/DNA template and does not act on single stranded RNA sequences.

Applicants note that claims 113-121 depend from claim 112. Therefore, arguments made with respect to claim 112 would be applicable to claims 113-121 as well.

5.1.2 Claims 133-142

The Office Action specifically states with respect to claim 133:

> With regard to Claim 133,the claim encompass all the limitations of Claim 91 and further that the nucleic acid which is copy is RNA and that a double stranded DNA template is formed in the process. Schuster et al. teaches a method of amplification in which the desired nucleic acid molecules can be RNA (Column 9, lines 52-53).
>
> Schuster et al. teaches a method of amplification in which a starting ssRNA analyte is primed in conditions for replication, a double stranded DNA template is produced, and RNase H is used to remove the RNA primers segment to allow the next priming event to occur (Figure 2).

Applicants respectfully traverse the rejection. Schuster does not contain each and every element of the claimed invention, the subject matter recited in claim 133.

Applicants note that step (e) has been amended in claim 133. For Examiner's reference, steps (d) and (e) of claim 133 as amended recites:

> (d) using said DNA copy as a template to produce a double-stranded copy comprising a second copy complementary to said DNA copy produced in step (c); and
>
> (e) removing said ribonucleic acid segment of said primers with RNase H from said double-stranded copy produced in step (d) **to regenerate a primer binding site on said template of (c) to render said primer binding site available for subsequent primer binding events** and producing more than one copy of said RNA of interest.

Schuster et al. use an RNA copy to make a cDNA copy. They only use RNase H after this cDNA copy has been made to produce a single-stranded cDNA molecule. After the RNase step, they use the cDNA copy to make a double-stranded molecule which is DNA/DNA and this is <u>not</u> followed by RNase H as required by step (e). In summary, and in contrast to the method of the present invention, Schuster et al. never remove an

RNA segment with RNase H from a double stranded copy (step d) made after a DNA copy is made from a DNA copy (step e). Even if Schuster had used a primer comprising an RNA segment, this would not have appeared in the transcription product of Schuster et al. and would also not be present in the cDNA copy of Schuster et al. There is never a discussion or suggestion of removal of RNA from primers, only digestion of RNA copies that have been generated from primer sequences.

Claims 134-142 depend from claim 133. Therefore, arguments made with respect to claim 133 would be applicable to claims 134-142 as well. Applicants do further note that the Examiner has stated that Schuster et al. teaches that the modified proto-primer can be RNA. Therefore, in the Examiner's view, it is inherent that the RNA strand contains deoxynucleotides.

Applicants however, would like to respond to one of the specific assertions made with respect to some of the dependent claims. It is stated with respect to claims 95, 116 and 137,

> With regard to Claims 95, 116,and 137, Schuster et al. teaches that the modified proto-primer can be RNA; therefore it is inherent that an RNA strand would be composed of deoxyribonucleotides (Column 11, lines 47-60).

Applicants disagree with these assertions. Specifically, a chimeric **primer** is not described in Schuster et al. Applicants concede that a strand made by extension of primer by a DNA polymerase does contain deoxyribonucleotides. However, step (b) recites "primer" not strand.

In view of the above arguments and amendments of claims 112 and 133, Applicants assert that the rejections over Schuster et al. under 35 USC 102 have been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

5.2 Kacian

Claims 91-97, 99-107, 109-118, 120-128, and 130-132 are rejected under 35 U.S.C. 102(e) as being anticipated by Kacian et al, (US Patent 5554516 September 10, 1996). Before addressing the rejection, Applicants note that claims 91-100 have been canceled in order to advance prosecution. Applicants do reserve the right to pursue

prosecution of the subject matter recited in the canceled claims in subsequent continuation and/or divisional applications.

5.2.1. Claims 112-118 and 120-122

The Office Action specifically states with respect to claim 112

> With regard to Claim 112, the claim encompasses the same steps as Claim 91 with the addition that an RNA/DNA hybrid substrate is produced and digested with RNase H to allow additional amplification events. Kacian et al. teaches that the target sequence and the promoter primer sequence will result in a DNA/RNA complex (Column 4, lines 40-45).

Applicants respectfully traverse the rejection. As with Schuster, Kacian et al. does not teach each and every element of the method of the present invention, the method recited in claim 112. It is Applicants view that Kacian et al. like Schuster does not teach step (d). As noted above, in Applicants method, only the primer portion of the substrate generated in step (c) is actually removed from the nucleic acid of interest so that a binding site is regenerated on said nucleic acid of interest. As a result, more primer binding and extension events from the nucleic acid of interest and original binding site occurs. In contrast, in Kacian, RNA copies are made off of the original template and these do not contain the primers themselves. Primer binding events only take place on resultant copies, **not at the original binding site**. In Kacian, the primer is not removed from the original template. Therefore, it would not be possible for another priming event to occur on the nucleic acid of interest using the method of Kacian.

Claims 113-118 and 120-122 depend from claim 112. Therefore, arguments made with respect to claim 112 would be applicable with respect to claim 113-118 and 120-122.

5.2.2 Claims 123-128 and 120-128

It is specifically asserted with respect to claim 123

> With regard to Claim 123, the claim encompasses the same steps as Claim 102 except that the RNA segment is removed by RNase H digestion. Kacian et al. teaches

> RNAse H degrades the RNA portion of RNA: DNA duplex (Column 8, lines 31-34).

Applicants respectfully traverse the rejection. It is Applicants assertion that Kacian does not recite each and every element of claim 123. Applicants note that step (d) of claim 123 has been amended to recite

> (d) removing said RNA segment of said primer from said template by digesting with RNase H to bind another primer to said template and initiate synthesis, thereby multiply initiating polynucleotide or oligonucleotide synthesis.

In Applicants' view, Kacian does not recite step (d) of claim 123. In Applicants method, only the RNA segment of said primers is actually removed from the template so that the binding site on the template is available for another primer binding event. As a result, more primer binding and extension events occur from the original binding site on the template. Specifically, in Kacian, primer binding events only take place on resultant copies, **not at the original binding site**. In Kacian, the primer is not removed from the original template. Therefore, it would not be possible to regenerate a primer binding site on the specific nucleic acid using the method of Kacian.

Claims 124-128 and 130-132 depend from claim 123. Therefore, arguments made with respect to claim 123 would apply to claims 124-128 and 130-132.

In view of the above arguments, Applicants assert that the rejection of claims 91-100, 112-121 and 133-142 under 35 USC 102 over Kacian has been overcome. Therefore, Applicants respectfully request that the rejection be withdrawn.

5.3 Cleuziat

Claims 91, 93-98, 102, 104-108, 112, 114-119, 123, 125-129, 133, and 135-142 have been rejected under 35 U.S.C. 102(b) as being anticipated by Cleuziat et al. (US Patent 5,824,517 October 20, 1998). It is stated in the Office Action that in the event that priority to previously filed applications is perfected then the rejection would be withdrawn. As noted above, priority has been perfected. Therefore, the rejection should be withdrawn.

6. The Rejections under 35 USC § 103

The rejections under 35 USC 103 are set forth below.

6.1 The Rejections over Schuster in view of Skerra

Claims 101 and 122 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Schuster et al. (US Patent 5,169,766 December 8, 1992) in view of Skerra (Nucleic Acids Research 1992Vol. 20 p. 3551). The Office Action specifically states:

> Schuster et al. teaches a method of amplifying a nucleic acid molecule. Schuster et al. teaches providing a DNA target and mixing the target with nucleoside triphosphates (nucleic acid precursors) (Figure 1 Column 7, lines 60-65). Schuster et al. teaches the proto-primer used can be a RNA sequence (Column 11, lines 47-51). Schuster et al. teaches the use of RNase H to remove RNA from the cDNA (Column 8,lines 7-10). Schuster et al. teaches an mRNA promoter (primer) which is used to extend and make ssRNA (Figure 3). Schuster et al. teaches another primer (DNA) is annealed to the ssRNA and cDNA is copied (Figure 3).
>
> Schuster et al. teaches making RNA: DNA hybrid (Column 9, lines 32-33). Schuster et al. teaches the ssRNA (which is the extended promoter) is destroyed by RNase H. Further, any primers which are in the solution but did not primer to the original ssDNA would be destroyed by RNaseH, therefore allowing for a reaction solution with only the cDNA that allows the completion of another cycle and the production of another cDNA strand identical to the ssDNA template.
>
> Schuster et al., however, does not teach primers modified by heteroatoms comprised of nitrogen or sulfur and chemically modified primers comprised of nucleoside triphosphates.
>
> Skerra teaches a method of using phosphorothioate primers in an amplification method (Abstract). With regard to Claims 96-97, Skerra teaches the modification of primers by the addition of a single phosphorothioate bond (heteroatom of sulfur) at the first 3' terminal internucleotide linkage during synthesis of the oligodexoynucleotide (p.

> 3552 1" column last paragraph). Skerra teaches that the phosphorothiate bond is much less favored substrate to nuclease activity than the naturally occurring phosphodiester bond (P, 3552 1" column last sentence and **2nd** column 1" sentence).
>
> Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Schuster et al., to use the phosphorothioate primers as taught by Skerra. The ordinary artisan would have been motivated to modify the method of Schuster et all because Skerra teaches the use of phosphorothiate primers would avoid the lower PCR yield and non-specific side products resulting from **3'** terminal editing of the primer molecule by protecting the oligodeoxynucleotide from a 3' terminal exonuleoytic attack (p. 3553 2nd column last paragraph).

Applicants respectfully traverse the rejection. First, as noted above, claim 101 has been canceled in order to advance prosecution.

Applicants note that claim 122 depends from claim 112. As argued above, Schuster et al. does not disclose the method recited in amended claim 112. It would not be obvious to combine the disclosures of Schuster et al. with Skerra. Schuster et al. is directed to amplification and Skerra is directed to oligonucleotide analogs. There was no suggestion regarding the use of analogs in the amplification method of Schuster. Furthermore, it is admitted in the Office Action that Schuster et al. does not disclose chemically modified primers.

Furthermore, as noted above, there is no disclosure of step (d) recited in claim 112 in Schuster et al. Therefore, even if the disclosures of Schuster et al. and Skerra are combined, the method of the present invention would not be obtained given the lack of disclosure of step (d). Even assuming *arguendo* that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Schuster et al., to use the phosphorothioate primers as taught by Skerra and/or the ordinary artisan would have been motivated to modify the method of Schuster et al., the claimed method would have not been obtained since step (d) is not disclosed.

In view of the above arguments, Applicants assert that the rejection of claims 101 and 122 under 35 USC 103 have been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

6.2 The Rejections of Kacian et al. in view of Schuster

Claims 98, 108, 119 and 129 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Kacian et al. (US Patent 5554516 September 10,1996) in view of Schuster et al. (US Patent 5,317,087 May 31, 1994). The Office Action specifically states:

> Kacian et al. teaches a method of amplifying a target nucleic acid sequence (Abstract). Kacian et al. teaches a method of incubating a promoter-primer and a target sequence in DNA priming and nucleic acid synthesizing conditions (ribonucleotide triphosphates and deoxyribonucleotidetriphosphates) (nucleic acid precursors) for a period of time to many multiple copies of the target sequence (Column 10 lines 23-33).
>
> Kacian et al. teaches the promoter-primer may be altered with ribonucleotides (Column 9, line 15).Therefore the promoter-primer can be RNA: DNA mixture. A primer, which includes a segment of RNA, would be encompassed by the promoter-primer. Kacian et al. teaches using a DNA polymerase (nucleic acid producing catalyst) (Column 10 line 59). Kacian et al. teaches that generation of target sequence is done using RNase H (Column 4 lines 65-67 and Column 5 lines 1-5).
>
> Kacian et al. teaches the promoter-primer can have both RNA and a DNA region (Column 9, line 15).
>
> Kacian et al. teaches that the target sequence and the promoter primer sequence will result in a DNA/RNA complex (Column4, lines 40-45).
>
> Kacian et al. teaches RNAse H degrades the RNA portion of RNA: DNA duplex (Column 8, lines 31-34).
>
> Kacian et al., however, does not teach the use of Taq DNA polymerase. Schuster et al. teaches a method of amplifying a nucleic acid molecule. With regard to Claims 98, 108, 119, and 129, Schuster et al. teaches providing a DNA target and mixingthe target with nucleoside triphosphates. Schuster et al. teaches DNA polymerase includes Taq polymerase, Klenow polymerase, E. coli polymerase, and T7 DNA polymerase (Column 7, lines 14-20).
>
> Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Kacian et al., to use Taq

> Polymerase as taught by Schuster et at. The ordinary artisan would have been motivated to modify the method of Kacian et al., because Schuster et al. teaches the use of Taq Polymerase is a preferred DNA polymerase (Column 7, lines 14-15). The ordinary artisan would want to use the most preferred polymerase for incorporating nucleoside triphosphates to extend the nucleic acid in order the perform an amplification method which can produce many copies of a target sequence.

Applicants respectfully traverse the rejection. Claim 98 depends from claim 91; claim 108 depends from claim 102; claim 119 depends form claim 112 and claim 129 depends from claim 123.

As argued above with respect to claims 112 and 123, Kacian et al. does not disclose step (d) of the claimed method, essentially, removal of ribonucleotides (RNA segment) from the nucleic acid sequence of interest (termed "template in claim 123) so that the original primer binding site is available for another primer binding event to occur. As noted above, Schuster would not fill in the gap, given that Schuster does not disclose step (d) either. Therefore, even assuming *arguendo* that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Kacian et al., to use Taq polymerase as taught by Schuster, the claimed method would have not been obtained, since step (d) could not be accomplished given the disclosures of both of these references.

In view of the above arguments, Applicants assert that the rejection of claims 98, 108, 119 and 129 under 35 USC 103 have been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

7. Double Patenting

Claims 91-101 provisionally rejected on the ground of nonstatutory obviousness- type double patenting as being unpatentable over claims 91-99 of copending Application No. 10/718391. In response, Applicants note that claims 91-99 have been canceled. Therefore, this rejection should be withdrawn.

8. Conclusion

In view of the foregoing, Applicants assert that the claims are now in condition for allowance. Early action to that end is respectfully requested. The Examiner is invited to contact the undersigned at (914) 712-0093 if she has any questions.

Respectfully submitted,

Date: 12/4/06

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